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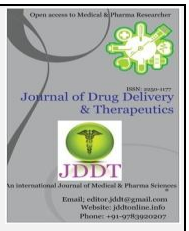

Open Access Research Article

METHOD DEVELOPMENT AND VALIDATION OF CLOPIDOGREL DRUG IN THE DRUG SUBSTANCES AND DOSAGE FORM BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The present work describes a validated reverse phase high performance liquid chromatographic method for estimation of Clopidogrel in the drug substances and dosage form. The quantification was carried out using Ultron ES OVM (150*4.6) mm, 5µm and mobile phase comprised of Buffer, Acetonitrile and in proportion of 70:30 %v/v. The flow rate was 1.0 ml/min and the eluent was monitored at 220 nm. The selected chromatographic conditions were found to effectively quantitate Clopidogrel at retention time of about 3.8 min. Linearity were found to be in the range of 11-75 µg/ml. The percentage recoveries of all the drugs were found to be 99.3-101.1%. The proposed method was found to be fast, specific, accurate, precise, and reproducible and can be used for estimation of the Clopidogrel drugs.

Keywords: Clopidogrel, Reversed-phase HPLC.

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INTRODUCTION

Clopidogrel is a prodrug, the action of which may be related to an adenosine diphosphate (ADP) receptor on platelet cell membranes. The drug specifically and irreversibly inhibits the P2Y subtype of ADP receptor, which is important in aggregation of platelets and cross-linking by the protein fibrin**.** Absorption and Distribution: It is rapidly absorbed after oral administration of repeated doses of 75 mg Clopidogrel, with peak plasma levels of the main circulating metabolite occurring approximately one hour after dosing. Absorption is at least 50% based on urinary excretion of Clopidogrel related metabolites.Clopidogrel undergoes rapid hydrolysis into its carboxylic acid derivative. In plasma and urine, the glucuronide of the carboxylic acid derivative is also observed.

MATERIALS AND METHODS

Reagents and Chemicals:

Clopidogrel drug sample, acetonitrile solvent of HPLC grade, potassium di-hydrogen phosphate, water of HPLC grade.

Instruments and Chromatographic Conditions:

HPLC system with UV/PDA detector was used for method development and validation. The separation were achieved on Ultron ES OVM (150*4.6) mm, 5µm column. The column was maintained at room temperature and the eluent was monitored at 220 nm using UV detector. The mixture of Potassium dihydrogen phosphate buffer 20mM (pH 4.2): Acetonitrile 70:30v/v at a flow rate of 1.0 ml/min was used as a mobile phase. The injection volume was 20µl.

Preparation of Mobile phase:

Potassium di-hydrogen phosphate buffer 20 mM (pH 4.2): Acetonitrile in the ratio of 70:30 v/v.

Preparation of diluent

Mixed Methanol and water in ratio of 90:10 (v/v)

Preparation of standard solution

Weighed about 75 mg of Clopidogrel standard into 50 mL volumetric flask. Added 30 mL of diluent, sonicated to dissolve and made up volume with diluent and mixed well. Further, 5 ml of above solution dilute to 200 ml with diluent.

Preparation of sample solution

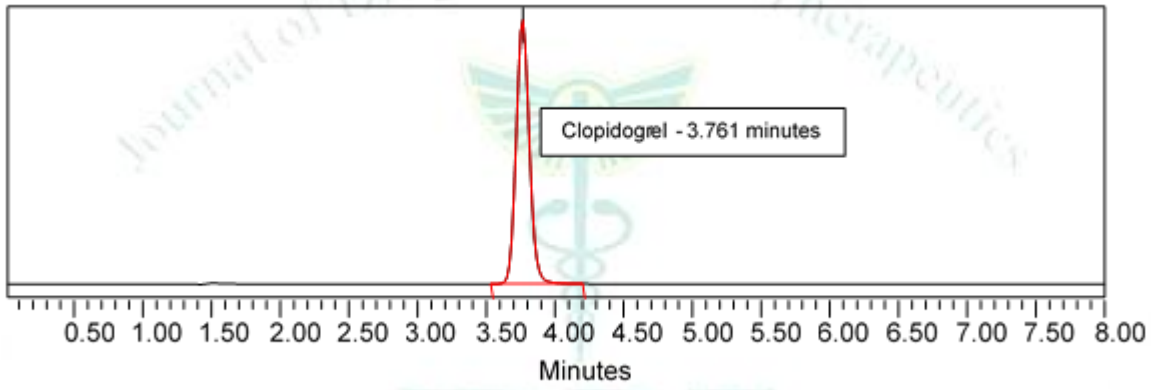
Weighed 20 tablets to find out average weight and crushed in to mortal pestle. Weighed the crushed sample equivalent to 375 mg of Clopidogrel and transferred in to 500 ml of volumetric flask. Added about 400 ml of diluent and sonicated for 60 minutes than made up volume with diluent and mixed well. Further, 5 ml of above solution dilute to 100 ml with diluent.

Chromatography parameter:

Column	Ultron ES OVM (150*4.6)mm,5µm
Sample temp.	25°C
Injection volume	20µL
Flow rate	1.0ml/min
Detector wavelength	220nm
Column temperature	25°C
Detector	UV
Diluent	BUFFER:ACN (70:30)
Mobile phase B	Acetonitrile
Mobile phase A	BUFFER pH 4.2
Run time	8 minutes

RESULTS AND DISCUSSION

Typical chromatogram of sample



Validation of RP-HPLC method

1 System precision

The % RSD for area response of six replicate injection for standard solution found to be 1.5 % which is within the acceptance criteria of 2.0%

No. of Injections	Area
1	2022230
2	2098623
3	2104221
4	2098231
5	2087221
6	2105561
Average	2086014.5
SD	31911.4
% RSD	1.5

2 Specificity

There are no any interference or peak observed from blank and placebo chromatogram at the retention time of Clopidogrel peak

3 Method precision

The % RSD for % assay of six replicate sample preparation were found to be 0.5 % which is within the acceptance criteria of 2.0%

No. of preparations	% Assay
1	98.7
2	99.3
3	99.1
4	98.1
5	99.2
6	99.5
Average	99.0
SD	0.5
% RSD	0.5

4 Accuracy

Accuracy were performed on 11 µg/ml (30%), 37.5 µg/ml (100%) and 75 µg/ml (200%). The mean % recovery found to be as below table.

Accuracy level	Concentration (µg/ml)	% Recovery	% Mean recovery
30% preparation-1	11 µg/ml	99.5	100.4
30% preparation-2		100.5	
30% preparation-3		101.1	
100% preparation-1	37.5 µg/ml	99.3	99.6
100% preparation-2		99.8	
100% preparation-3		99.6	
200% preparation-1	75 µg/ml	100.4	100.1
200% preparation-2		99.6	
200% preparation-3		100.2	

5 Linearity

Linearity were performed on 5 concentration level considering 11 µg/ml (30%) to 75 µg/ml (200%). The correlation coefficient found to be 1.000.

S.No.	Linearity Level	Concentration	Area response
1	Linearity at 30%	11.1	604343
2	Linearity at 50%	18.8	1099112
3	Linearity at 100%	37.6	2098899
4	Linearity at 150%	56.3	3124153
5	Linearity at 200%	75.1	4109856
Correlation coefficient		1.000	

6 Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.

2. pH of Mobile phase was changed (± 0.2) 4.0 & 4.4.

3. Ratio of Mobile phase was changed (±2) Buffer: Acetonitrile (72:28) and Buffer: Acetonitrile (68:32)

The system suitability parameter was found to be within the acceptance criteria in all above conditions.

CONCLUSION

From the above discussion it can be concluded that the proposed method is specific, precise, accurate, linear and robust. Results are in good agreement with label claim which indicates there is no interference of excipients. Therefore the proposed method can be used for routine analysis of Clopidogrel in drug substances and formulation.

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